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Novel derivatives of ISO-1 as potent inhibitors of MIF biological function

Sarala Balachandran [a,*], Atish Rodge [a], Pradip K. Gadekar [a], Vitthal N. Yadav [a], Divya Kamath [b], Anshu Chetrapal-Kunwar [b], Pooja Bhatt [b], Shaila Srinivasan [b], Somesh Sharma [a,b], Ram A. Vishwakarma [a], Nilesh M. Dagia [b]

[a] Department of Medicinal Chemistry, Piramal Life Sciences, 1 Nirlon Complex, Off Western Express Highway, Goregaon (E), Mumbai 400 063, India
[b] Department of Pharmacology, Piramal Life Sciences, 1 Nirlon Complex, Off Western Express Highway, Goregaon (E), Mumbai 400 063, India

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ABSTRACT

A series of novel 1,2,4-oxadiazole, phthalimide, amide and other derivatives of ISO-1 were synthesized and probed for inhibition of macrophage migration inhibitory factor (MIF) activity. Several compounds inhibited MIF enzymatic activity at levels better than ISO-1. Of note, compounds **7**, **22**, **23**, **24**, **25** and **27** inhibited the spontaneous secretion/release/recognition of MIF from freshly isolated human peripheral blood mononuclear cells and, more importantly, inhibited the MIF-induced production of interleukin-6 (IL-6) and/or interleukin-1β (IL-1β) significantly better than ISO-1.

A growing body of evidence from in vitro, in vivo and, most importantly, clinically relevant studies implicates the pleiotropic pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) in the pathogenesis of a variety of inflammatory and autoimmune diseases.[1,2] Therefore, a promising therapeutic approach to diminish pathological inflammation is to inhibit the production and/or biological activity of MIF. Given that MIF possesses enzymatic properties,[1,2] combined with the observations that inhibiting the catalytic site of MIF leads to a marked reduction in the biological function of MIF,[3] an attractive pharmacological strategy to inhibit inflammation is to target the catalytic pocket of MIF.[2]

One of the earliest reported small molecule catalytic MIF inhibitor was from the isoxazole series.[3] ISO-1, the pseudo-lead molecule from this series (Fig. 1), inhibits the macrophage release of TNF-α from LPS-stimulated mice and is moderately protective in a clinically relevant model of sepsis when administered intraperitoneally.[3] In a quest towards increasing the potency of ISO-1, modifications were carried out on the ISO-1 scaffold. Synthesis and biological activity evaluation of derivatives of ISO-1 revealed that mono fluorination ortho to the phenolic group of ISO-1 increased the activity profile of the analogs[4] and that amides of ISO-1 were comparatively less active at inhibiting dopachrome tautomerase activity.[4] Given the observations of this earlier study, we decided to synthesize fluorinated analogs of ISO-1 introducing heterocycle on isooxazole ring and/or making reverse amides. This led to the synthesis of novel series of 1,2,4-oxadiazoles, phthalimide and amide derivatives of ISO-1.

The key intermediates 2-(3-(-3-fluoro-4-methoxyphenyl)-4,5-dihydroisoxazole-5-yl)-*N*′-hydroxyacetimidamide (**5**) and 3-(-3-fluoro-4-methoxyphenyl)-*N*′-hydroxy-4,5-dihydroisoxazole-5-carboximidamide (**9**) required for synthesis of the 1,2,4-oxadiazoles were synthesized utilizing a four-step reaction starting with 3-fluoro-4-methoxy benzaldehyde.[5,6] 3-Fluoro-4-methoxy benzaldehyde (**1**) was treated with hydroxylamine hydrochloride in the presence of triethyl amine to yield the desired oxime (**2**)[4] in 95% yield. The oxime (**2**) was further treated with *N*-chlorosuccinimide in DMF resulting in the formation of corresponding chloro-oxime (**3**), which was treated in situ with allyl cyanide in presence of triethyl amine to yield 2-(3-(3-fluoro-4methoxyphenyl)-4,5-dihydroisoxazol-5-yl) acetonitrile (**4**) in 85% yield. Compound **4** was further reacted with hydroxylamine hydrochloride in the presence of sodium carbonate in ethanol–water to yield 73% of the desired key intermediate **5**[5] (Fig. 2). With the objec-

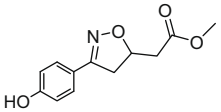

Figure 1. ISO-1, one of the earliest reported MIF inhibitor.

* Corresponding author.
E-mail address: sarala.balachandran@piramal.com (S. Balachandran).

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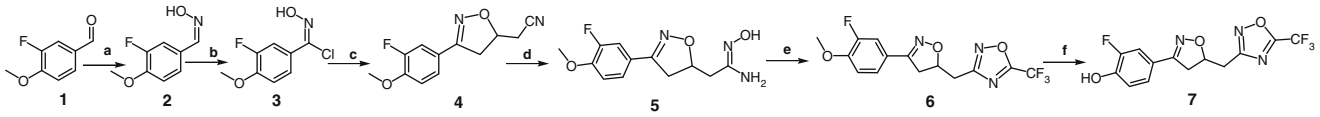

Figure 2. Synthesis of compounds **2**–**7**. Reagents and conditions: (a) $NH_2OH{\cdot}HCl/Et_3N/CH_2Cl_2$, rt, 15–16 h, 95%; (b) NCS/DMF, rt, 4–5 h; (c) allyl cyanide/Et_3N, rt, 24–26 h, 85%; (d) $NH_2OH{\cdot}HCl$, Na_2CO_3/EtOH, H_2O, rt, 6 h, 73%; (e) trifluoroacetic anhydride/pyridine, 100–120 °C, 8 h, 60%; (f) BF_3–DMS/DCM, 78 °C, 15–16 h, 41%.

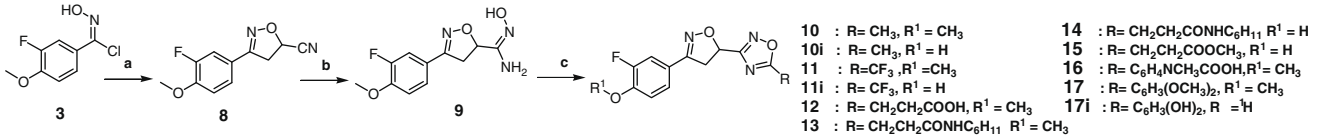

Figure 3. Synthesis of compounds **8**–**17i**. Reagents and conditions: (a) acrylonitrile/Et_3N, rt, 24–26 h, 93%; (b) $NH_2OH{\cdot}HCl$, Na_2CO_3/EtOH, H_2O, rt, 6 h, 82%; (c) (**10**) acetic anhydride, pyridine, 100–120 °C, 8 h, 64%; (**10i**) compound **10**, BF_3–DMS/DCM, −78 °C, 15–16 h, 53%; (**11**) compound **9**, trifluoroacetic anhydride, pyridine, 100–120 °C, 8 h, 61%; (**11i**) compound **11**, BF_3–DMS/DCM, −78 °C, 15–16 h, 53%; (**12**) compound **9**, succinic anhydride/pyridine, 100–120 °C, 8 h, 64%; (**13**) compound **12**, $SOCl_2$, 40 °C, 1 h, Et_3N, cyclohexylamine, rt, 8 h, 80%; (**14**) compound **13**, BF_3–DMS/DCM, −78 °C, 15–16 h, 53%; (**15**) compound **12**, BBr_3/DCM, −78 °C, 15–16 h, 60%; (**16**) compound **9**, *N*-methylisatoic anhydride/pyridine, 100–120 °C, 8 h, 67%; (**17**) compound **9**, CDI/DMF, 2-3-dimethoxy benzoic acid, rt, 1–2 h, 80–100 °C, 6–7 h, 75%; (**17i**) compound **17**, BF_3–DMS/DCM, −78 °C, 15–16 h, 56%.

tive of introducing a heterocycle on isoxazole ring, initially, intermediates (**5** and **9**) were reacted with different anhydrides (trifluoroacetic anhydride, acetic anhydride, succinic anhydride, *N*-methylisatoic anhydride) using pyridine at 80–110 °C to yield substituted 1,2,4-oxadiazoles (**6**, **10**, **11 and 16**, respectively) (Figs. 2 and 3). In an alternative approach (to introduce a heterocycle on isoxazole ring), intermediate **9** was reacted with 2,3-dimethoxy benzoic acid using CDI in DMF at 80–100 °C for 6–7 h. to afford **17** in 75% yield. To dissect the contribution of 'methylation' to the observed MIF inhibitory activity, demethylated analogs of several compounds were synthesized (e.g., **10i**, **11i**, **14**, **15**, **17i**) using BF_3–DMS. The reduction of nitrile compounds **4** and **8** was carried out using hydrogenation in presence of 10% Pd–C in methanol which resulted in the isolation of amines **18i** and **18**, respectively (Figs. 4 and 5). Subsequently, the amide derivatives of ISO-1 were synthesized by treating these isoxazoline amines with different acyl or aryl acid halides (pivaloyl chloride, isovaleryl chloride and *N*,*N*-dimethlycarbamoyl chloride) in the presence of triethylamine. After demethylation the reverse amides (**19–25**) were obtained (Figs. 4 and 5). The phthalimide derivatives of ISO-1 (**26–29**) were synthesized using oxime **2** in cycloaddition reaction with various *N*-alkene phthalimides in the presence of NCS or sodium hypochlorite (Fig. 6) followed by demethylation. The structures of various synthesized compounds were assigned on the basis of different spectral data (experimental details are provided in Supplementary data).

We investigated the MIF inhibitory potential of the synthesized compounds and, in parallel, compared their activity with ISO-1. Initially, dopachrome tautomerase assays were performed. Several compounds from both 1,2,4-oxadiazole series (e.g., **7**, **12**, **13**, **14**, **17 and 17i**) and phthalimide and amide derivatives of ISO-1 (e.g., **22**,

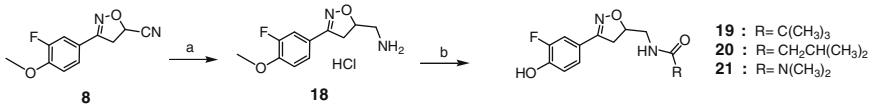

Figure 4. Synthesis of compounds **18**–**21**. Reagents and conditions: (a) H_2, Pd/C, MeOH, rt, 2 h, 63% (b) (**19**) (i) pivaloyl chloride, Et_3N, THF, rt, 2 h; (ii) $BF_3{\cdot}$DMS/DCM, −78 °C, 15–16 h, 37%; (**20**) isovaleryl chloride, Et_3N, THF, rt, 2 h; (ii) BF_3–DMS/DCM, −78 °C, 15–16 h, 47%; (**21**) (i) *N*,*N*-dimethyl carbamoyl chloride, Et_3N, THF, rt, 2 h; (ii) BF_3–DMS/DCM, −78 °C, 15–16 h, 18%.

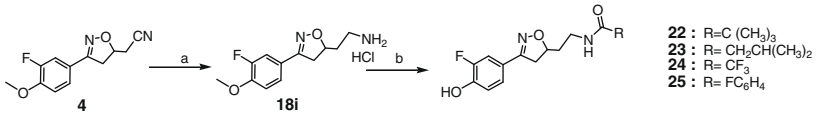

Figure 5. Synthesis of compounds **18i**–**25**. Reagents and conditions: (a) H_2, Pd/C, MeOH, rt, 2 h, 60% (b) (**22**) (i) pivaloyl chloride Et_3N, THF, rt, 2 h; (ii) BF_3–DMS/DCM, −78 °C, 15–16 h, 18%; (**23**) (i) isovaleryl chloride, Et_3N, THF, rt, 2 h; (ii) BF_3–DMS/DCM, −78 °C, 15–16 h, 22%; (**24**) (i) trifluoro acetic anhydride, Et_3N, THF, rt, 2 h; (ii) BF_3–DMS/DCM, −78 °C, 15–16 h, 9%; (**25**) (i) 4-fluoro, benzoyl chloride, Et_3N, THF, rt, 2 h; (ii) BF_3–DMS/DCM, −78 °C, 15–16 h, 8%.

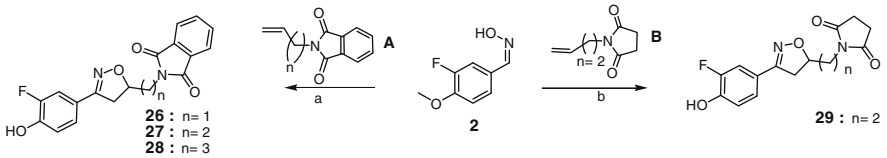

Figure 6. Synthesis of compounds **26**–**29**. Reagents and conditions: (**a**) (i) NCS, DMF, **A**, Et_3N or sodium hypochlorite (bleach), THF, rt, 48 h; (ii) BF_3–DMS/DCM, −78 °C, 15–16 h, 16%; (b) NCS, DMF, **B**, Et3N, rt, 15 h; (ii) BF_3–DMS/DCM, −78 °C, 15–16 h, 48%.

Table 1
Potency of synthesized 1,2,4-oxadiazoles to inhibit MIF enzymatic activity in tautomerase assay

Compd	IC_{50} (μM)	
	THP-1 cell lysates	Purified human MIF
ISO-1	69	>100
6	>100	55
7	48	60
10	>100	ND
10i	60	>100
11	100	95
11i	60	>100
12	12	>100
13	30	9.5
14	25	>100
15	50	>100
16	>100	ND
17	20	32
17i	19	>100

ND, not done.

Table 2
Potency of synthesized phthalimide and amide derivatives of ISO-1 to inhibit MIF enzymatic activity in tautomerase assay

Compd	IC_{50} (μM)	
	THP-1 cell lysates	Purified human MIF
ISO-1	69	>100
19	>100	ND
20	>100	ND
21	>100	ND
22	28	22
23	29	22
24	49	>100
25	15	20
26	29	36
27	0.5	0.8
28	2	7
29	15	50

ND, not done.

Table 3
Activity of synthesized compounds to inhibit the secretion/release/recognition of MIF in ELISA assays using hPBMCs

Compound	IC_{50} (μM)
ISO-1	25
7	15
12	60
13	Not active
14	Not active
15	Not active
17	Not active
17i	Not active
22	15
23	20
24	15
25	10
26	Not active
27	1.5
28	Not active
29	20

23, **24**, **25**, **26**, **27**, **28 and 29**) consistently inhibited the dopachrome tautomerase activity in THP-1 lysates at levels better than that of ISO-1 (Tables 1 and 2). Only compounds that inhibited the tautomerase activity in THP-1 lysates at levels equal to or better than ISO-1 were further screened for their enzyme inhibition potential using purified recombinant human MIF. Again, compounds **7**, **13**, **17**, **22**, **23**, **25**, **26**, **27**, **28** and **29** inhibited the tautomerase activity of purified recombinant human MIF at levels better than that of ISO-1 (Tables 1 and 2). These latter results (with purified human MIF) confirm the specificity of these novel 1,2,4-oxadiazoles, phthalimide and amide derivatives of ISO-1 to inhibit MIF enzymatic activity. Note that the difference in the observed and reported[3,4] IC_{50} value for ISO-1 in dopachrome tautomerase assay may be due to the different concentrations of purified human MIF utilized in the two studies.

In spite of the growing interest in inhibiting MIF activity via its catalytic pocket, the physiological relevance of the enzymatic activity of MIF is yet to be elucidated.[1,2] Based on our recent findings,[7] we sought to find compounds that inhibit not only the enzymatic activity of MIF but also (more importantly) the secretion/recognition and biological function of MIF. To this end, initially, only compounds that suppressed the enzymatic activity of MIF at levels equal to or better than that of ISO-1 were evaluated for their potential to inhibit the secretion/recognition of MIF from human peripheral blood mononuclear cells (hPBMCs). One compound from 1,2,4 oxadiazole series (i.e., **7**) and several phthalimide and amide derivatives of ISO-1 (e.g., **22**, **23**, **24**, **25**, **27** and **29**) inhibited the secretion/release/recognition of MIF from hPBMCs at levels equal to or better than ISO-1 (Table 3).

The secretion of MIF from THP-1 cells is known to occur via a non-classical pathway.[8] Reagents modulating the ABC transporter, but not protein synthesis inhibitors such as cycloheximide, are known to inhibit the secretion of MIF.[8] Whether ISO-1 and **7**, **22**, **23**, **24**, **25**, **27**, **and 29** work in a similar manner and inhibit the secretion of MIF by affecting the ABC transporter is currently unknown and warrants further investigation. However, the data generated from our recent study[7] and earlier studies[9] are consistent with the hypothesis that the inhibitory activity of ISO-1 and novel 1,2,4-oxadiazoles, phthalimide and amide derivatives of ISO-1 in cell-based ELISA assays is, at least in part, due to these compounds binding directly to MIF and preventing its recognition. Since several of these compounds (e.g., **7**, **22**, **23**, **24**, **25**, **27**, **and 29**) have a similar or lower IC_{50} for MIF inhibition in cell-based ELISA assays (in comparison to ISO-1), it would be of interest to determine whether these compounds have an equivalent or stronger affinity to interact with MIF in comparison to ISO-1.

It is well-known that tautomerase activity inhibitors down-regulate the production of pro-inflammatory cytokines.[3] Given that MIF induces the expression of pro-inflammatory cytokines,[1,2] we investigated if these compounds inhibit the purified human MIF-induced expression of TNF-α, IL-6 and IL-1β from hPBMCs. Only compounds that inhibited the enzymatic activity of MIF and also inhibited the secretion/release/recognition of MIF (at levels equal to or better than ISO-1) in ELISA assays were tested in these assays. Pre-treatment of MIF with compounds **7**, **22**, **23**, **24**, **25 and 27** led to a marked suppression in the induced production of IL-6 and/or IL-1β which was significantly better than ISO-1 (Table 4). Pre-treat-

Table 4
Potency of synthesized compounds to inhibit MIF-induced cytokine production from hPBMCs

Compd	(% Inhibition at 100 μM)[a]		
	TNF-α	IL-1β	IL-6
ISO-1	25 ± 25	25 ± 2.5	0 ± 0
7	34 ± 8.5	46 ± 0*	33 ± 5.5*
22	40 ± 10	50 ± 16	39 ± 8.5*
23	53 ± 6	58 ± 15	47 ± 5.5*
24	53 ± 12	66 ± 3.5*	49 ± 13
25	56 ± 0	52 ± 8*	54 ± 0.5*
27	31 ± 11	22 ± 2	22 ± 2*
29	39 ± 9	27 ± 26.5	14 ± 14

[a] Values are means ± S.E.M. from at least two separate experiments.
* Indicates $p < 0.05$ compared to ISO-1 control.

ment of MIF with **29** led to a relatively moderate, albeit similar to ISO-1-mediated, suppression in the induced production of TNF-α, IL-6 and IL-1β (Table 4). These data clearly demonstrate that, at least a few, 1,2,4-oxadiazoles and phthalimide and amide derivatives of ISO-1 inhibit the biological function of MIF at levels better than ISO-1. Collectively, these results provide evidence that novel derivatives of ISO-1 not only inhibit the enzymatic activity of MIF, but also, more importantly, inhibit the secretion/release/recognition and biological function of MIF.

In summary, a number of derivatives of ISO-1 were synthesized and screened for MIF inhibitory activity. In the enzymatic assays, it was observed that the demethylated analogs were more active than the corresponding methoxy analogs (e.g., **11i** vs **11**). In prior studies in the literature, the X-ray crystal structure of MIF and binding domains have been discussed.[3] To diversify our chemistry, we choose to complement and extend the findings of those prior studies. In all cases the phenol part of the ISO-1 scaffold was kept constant as the ortho fluoro phenol substituted isoxazoline was found to be favorable for binding in the MIF active pocket. Upon making modifications to the ester moiety of the ISO-1 scaffold, it is evident that flexibility is preferred in this region. Indeed, potent MIF inhibitory activity is observed with the phthalimide and amide derivatives of ISO-1. In contrast, MIF inhibitory activity is relatively absent from the heterocyclic derivatives. Of note, as would be hypothesized, variations in chain length give rise to variation in MIF inhibitory activity of synthesized compounds. It appears from our data that a chain length of $n = 2$ is optimum for MIF inhibitory activity (e.g., **23** and **25**).

Interestingly, several compounds from 1,2,4 oxadiazole series (e.g., **13**, **14**, **17**, **17i**) as well as phthalimide and amide derivatives of ISO-1 (e.g., **26** and **28**) which inhibited the enzymatic activity of MIF, did not inhibit the spontaneous secretion/release/recognition of MIF from hPBMCs. Compound **29** inhibits the enzymatic activity of MIF and also suppresses the spontaneous secretion/release/recognition of MIF from hPBMCs. However, **29** only moderately inhibits the biological function of MIF. Combined, the results of our study reveal that not all tautomerase activity inhibitors are equally efficacious in blocking the secretion of MIF and/or the biological function of MIF. It is tempting to speculate that the conformational changes of MIF caused by inhibitors binding to the enzymatic pocket[9] may contribute to differential potencies in biological assays. Clearly, future studies are warranted to completely understand the relationship between potential interactions (of various compounds) with the binding pocket of MIF and the resultant effects on biological function of MIF.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.052.

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